EFFECTS OF TWO ANTIBIOTICS ON NUCLEAR DIVISION OF *CHARA BRAUNII* Gm. (CHARACEAE)

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ABSTRACT - Effects of two antibiotics viz. streptomycin and chloramphenicol have been analysed on the nuclei of spermatocysts of *Chara braunii* Gm. Chloramphenicol is more inhibitory towards survival pattern than streptomycin. Cytological abnormalities like clumping of chromosomes, chromosomal fragments, micronuclei, anaphasic bridges and degenerate nuclei have been recorded. Lower concentrations of chloramphenicol cause duplication of chromosomes when compared to streptomycin doses.

RÉSUMÉ - Les effets de deux antibiotiques (streptomycine et chloramphenicol) sur les noyaux de spermatocystes de *Chara braunii* Gm. sont analysés. Le chloramphenicol montre une action inhibitrice plus forte sur le modèle de survie que la streptomycine. Des anomalies cytologiques (chromosomes agglutinés ou fragmentés, micronoyaux, ponts anaphasiques, noyaux dégénérés) sont observées. Des concentrations de chloramphenicol plus faibles que celles de streptomycine provoquent la duplication des chromosomes (traduit par la rédaction).

KEY WORDS : antibiotics, nuclear division, spermatocyst, *Chara braunii*, Characeae.

INTRODUCTION

As far as the authors are aware, the very first work on record pertaining to the effects of antibiotics on algal nuclear karyology is by Vedajanani & Sarma (1978) on *Spirogyra azygospora* Singh. Soon after, a few more algal members viz. *Oedogonium gunnii* Wittr. and *Spirogyra paradoxa* Rao have been subjected to antibiotic treatment by Srivastava & Sarma (1 980) and Abhayavardhani & Sarma (1981, 1982). Singh *et al.* (1988) have analysed penicillin mutagenecity in *Chara wallichii* A. Br.

Effects of antibiotics on various aspects of certain algae have been studied by numerous workers viz. Provasoli *et al.* (1948), Kumar (1964), Taylor (1965), Nora *et al.* (1965), Sager & Taubo (1961), Watanabe & Yamamoto (1968), Sri-

Name of antibiotic	Concentrations used(%)	Duration of treatment(hr.)	Post treatment fixation(hr.)	Cell Division(%)	Bi-nucleate Cells(%)	Cytological abnormalities
Streptomycin	Control	-	-	50	nil	Normal mitotic Process
	0.25	2,4	24	25	[illegible]	Bi-nucleate cells, degenerate nuclei, clumping of chromosomes and anaphasic bridges
			48	22	10	Identical observations as above
	0.5	2,4	24	15	12	Binucleate cells, micronuclei and degenerate nuclei
			48	10	15	Chromosome fragments and anaphasic bridges
	0.75	2,4	24	10	15	Duplication of Chromosome number and bi-nucleate cells
			48	7	17.5	Identical observations as above excepting formation of tri-nucleate cells.
	1.0	2,4	24	5	20	Duplication of chromosome number and bi-nucleate cells
			48	5	25	Unequal distribution of chromosomes
Chloramphenicol	Control	-	-	50	nil	Normal mitotic process
	0.25	2,4	24	37.5	15	lag-chromosomes, clumped metaphase plates, duplication of chromosome number bi-nucleate cells
	0.5	2,4	24	30	20	Duplication of chromosome number, degenerate nuclei, lobed nuclei and clumping of chromosomes
	0.75	2,4	24	nil	nil	Degeneration of spermatogenous filaments
	1.0	2,4	24	nil	nil	Degeneration of spermatogenous filaments.

Table I - Effects of streptomycin and chloramphenicol on the karyology of *Chara braunii*.

vastava & Nizam (1969, 1974), Reddy (1977), Puri & Grover (1980), Sathaiah & Vidyavati (1983) and Rao (1984).

Quite a limited work is available on the effects of antibiotics on chromosomes of higher plants and animals viz. Wilson (1950), Tanaka & Sato (1952), Sharma & Bhattacharyya (1967), Parida & Manna (1967) and Yoshid a *et al.* (1972).

On the basis of available literature, it appears that none of the members of Charophyceae have been subjected to antibiotic treatments excepting that of *Chara wallichii* by Singh *et al.* (1988). Keeping this in view, the present authors have undertaken a detailed study of the effects of streptomycin and chloramphenicol on the survival pattern and karyology of *Chara braunii* Gm.

MATERIAL AND METHODS

The alga was collected in fruiting condition from rice-fields near Pepee Compound area of Ranchi (India) in January, 1984. The cultures were maintained in laboratory at 21 ± 1°C exposing them to 16 hours light and 8 hours dark periods. Tap water was used as culture medium.

Different concentrations vz. 0.25%, 0.5%, 0.75% and 1.0% of streptomycin (Alembic Chemicals, India: 1gm streptomycin sulphate) and chlororamphenicol (Dey's India: entromycetin intramuscular) were prepared in distilled water.

A few mature plants with sex organs were subjected to each grade of antibiotic treatment ranging from 2 to 4 hours. The treated materials were then thouroughly washed in water and finally were put in tap water cultures to grow in the laboratory.

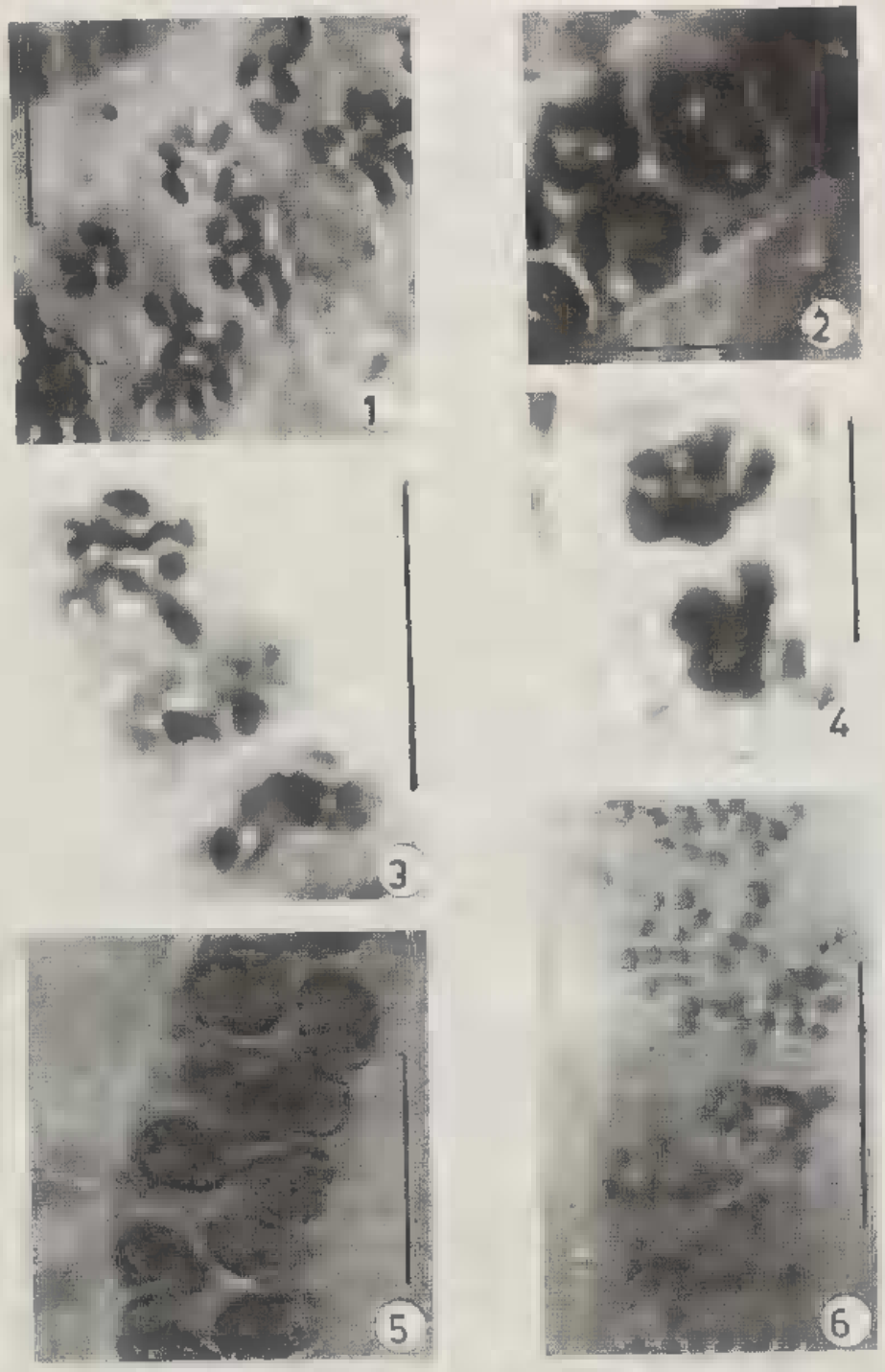
The treated materials of different concentrations as well as the control materials were fixed in acetic alcohol in the ratio of 1:3 for karyological studies. In case of streptomycin, fixations were made after 24 and 48 ho urs of treatment while such fixations were made after 24 hours only in respect with chloramphenicol. Godward's (1948) acetocarmine squash cytological schedule was followed in the present investigation.

Cell division frequency in spermatogenous cells was determined for each concentration as well as for the control by counting the number of cells in division stages and the number of cells in interphase condition.

OBSERVATIONS

A chromosomal count of n = 14 has been determined in the control specimen of *Chara braunii* (Fig. 7). Various stages of normal mitotic division without abnormalities have been recorded and about 50% spermatogenous cells have been found in dividing stages.

Effects of streptomycin and chloramphenicol as encountered during the present analysis are described below in sequence while their details are presented in Table I.



Figures 1 to 6: Effects of streptomycin on nuclear divisions in *Chara braunii* Gm. - Fig. 1: unequal distribution of chromosomes at metaphase. Fig. 2: degenerate and vacuolate nuclei. Fig. 3: anaphasic bridge. Fig. 4: clumped metaphase plates. Fig. 5: bi- and tri-nucleate cells. Fig. 6: duplication of chromosome number. Scale bars: 10μm.

A. Streptomycin:

Materials treated with 0.25% and 0.5% concentrations are healthy even after 5 days of treatment while materials treated with 0.75% and 1.0% concentrations turn pale and die down after 5 days.

Frequency of cell division is inhibited with the increase of doses (Table I and Fig. 12).

Bi-nucleate cells are observed in all concentrations (Fig. 5) and their percentage increases with the doses (Fig. 13). 0.75% concentration causes the formation of tri-nucleate cells (Fig. 5).

0.75% and 1.0% concentrations have been found to induce doubling of chromosomes viz. 28 chromosomes (Fig. 6).

Cytological abnormalities like clumping of chromosomes (Fig. 4), micronuclei, chromosome fragments, anaphasic bridges (Fig. 3) and degenerate and vacuolate nuclei (Fig. 2) have been encountered as the effects of different doses of this antibiotic when the material was fixed after 24 hours of treatment.

In addition to the effects noted with 24 hours post-treatment fixation, the formation of tri-nucleate cells (Fig. 5) and unequal distribution of chromosomes (Fig. 1) have also been recorded with 48 hours post-treatment fixation.

B. Chloramphenicol:

Materials treated with 0.25% and 0.5% concentrations turn pale in colour after 48 hours of treatment. However, 0.75% and 1.0% concentrations are found to be lethal which lead to degeneration of spermatogenous filaments.

Percentage of cell division decreases with the increase of doses of this drug (Fig. 12 and Table I).

0.25 and 0.5% concentrations have been found to induce the formation of bi-nucleate cells (Fig. 10) and duplication of chromosome number viz. 28 chromosomes (Fig. 9).

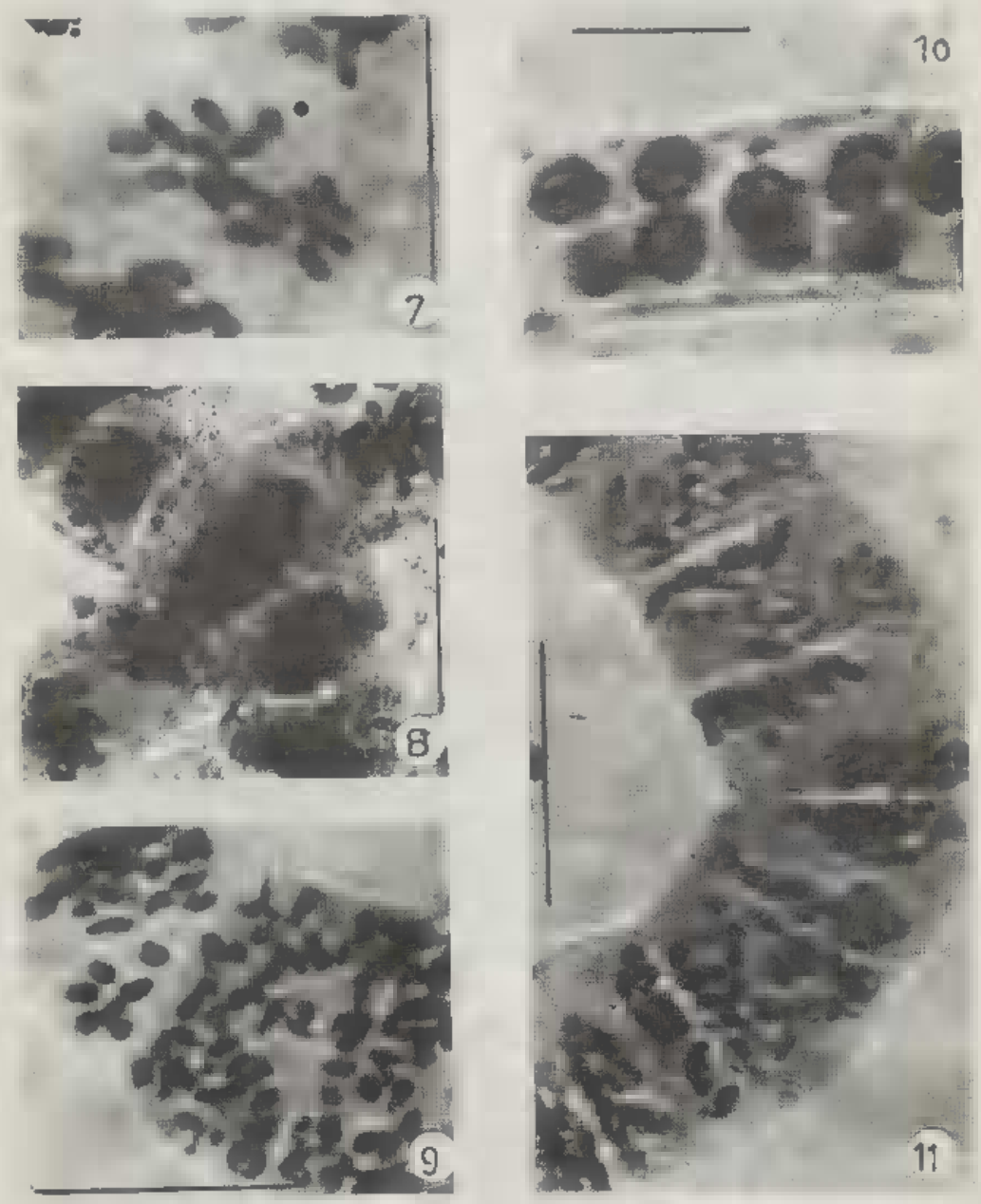
There are certain cytological aberrations viz. formation of clumped metaphase plates (Fig. 8), degenerate nuclei, laggards (Fig. 11) and bi-nucleate cells (Fig. 10) which have been observed when the plant material is treated with 0.25% and 0.5% concentrations.

DISCUSSION

Provasoli *et al.* (1948) and Nora *et al.* (1965) have noted the inhibitory effect of streptomycin in context with the formation of chlorophyll pigments in *Euglena*. Effect of identical nature has also been recorded by Sathaiah & Vidyavati (1983) in *Cosmarium praemorsum* Breb. Kumar (1964) has reported inhibition of growth and pigment production while Reddy (1977) has analysed its toxicity in blue-green algae.

In the present investigation, streptomycin appears to be inhibitory pertaining to growth pattern and frequency of cell division. Rao (1984) has reported inhibitory nature of this drug towards survival pattern as well as percentage of cell di-

vision in *Sirogonium phacosporum* Skuja. The percentage of cell division in the present study decreases with the increase of doses for this drug (Fig. 12). Rao



Figures 7 to 11: Effects of chloramphenicol on nuclear divisions in *Chara braunii* Gm. - Fig. 7: normal count (n = 14) in control material. Fig. 8: clumped metaphase plates. Fig. 9: duplication of chromosome number. Fig. 10: bi-nucleate cells. Fig. 11: anaphases with laggards. Scale bars: 10μm.

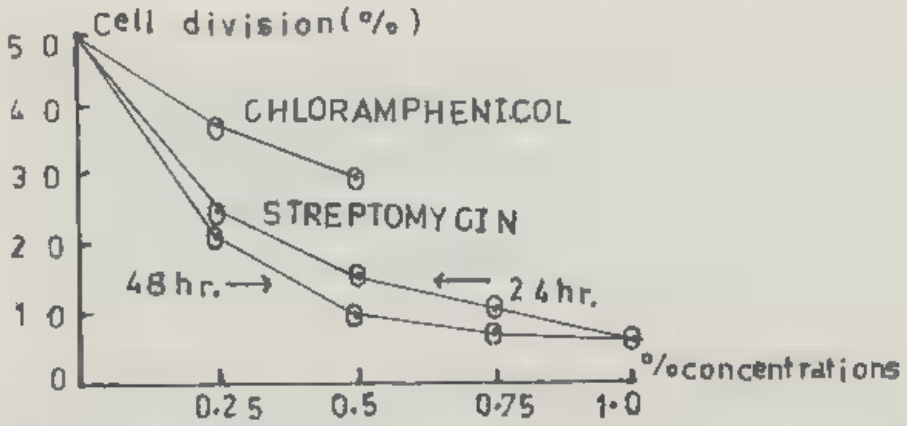


Fig. 12: Effects of different concentrations of chloramphenicol and streptomycin on the percentage of cell division in *Chara braunii* Gm.

(1984) records similar observation while treating *Sirogonium phacosporum* with this antibiotic.

It is obvious in the present work that lower doses viz 0.25% and 0.5% of chloramphenicol are inhibitory towards growth pattern while higher doses viz 0.75% and 1.0% are exclusively lethal. Taylor (1965) has noticed the inhibitory effect of this drug on the growth of *Scenedesmus quadricauda* (Turp.) Breb. Its toxic and inhibitory nature have also been recorded by Vedajanani & Sarma (1978) in *Spirogyra azygospora* in context to its survival pattern and by Puri & Grover (1980) in *Anabaena* and *Cylindrospermum* towards heterocyst formation.

The percentage of cell division decreases with the increase of doses in case of chloramphenicol (Fig. 12) as envisaged in the present investigation. However, Vedajanani & Sarma (1978) report mitotic delay in *Spirogyra azygospora* as the culminating effect of this drug.

As per our study, chloramphenicol has been proved more inhibitory towards survival pattern than streptomycin, whereas frequency of cell division has been inhibited to a greater extent by streptomycin than chloramphenicol (Fig. 12).

The formation of bi-nucleate cells (Fig. 5) has been induced by almost all the doses of streptomycin employed, while 0.75% of its concentration induces the formation of tri-nucleate cells (Fig. 5). Identical effects have also been recorded previously by Rao (1984) while treating *Sirogonium phacosporum* with this antibiotic.

Vedajanani and Sarma (1978) have noted the formation of bi- and tri-nucleate cells for the first time in *Spirogyra azygospora* after treatment with chloramphenicol. However, bi-nucleate cells (Fig. 10) have been observed, when treated with 0.25% and 0.5% concentrations of this drug.

Duplication of chromosomes viz. 28, (Fig. 6 and Fig. 9) has been induced by lower doses viz. 0.25% and 0.5% of chloramphenicol while higher doses of

streptomycin viz. 0.75% and 1.0% are required for identical effect. The duplication of chromosomes number by employing these antibiotics, has not been recorded previously in any plant. However, colchicine has been widely used to induce doubling of chromosomes in plants and animals as well as in a number of algal members viz. in *Oedogonium acmandrium* Elfing by Sarmaz & Tripathi (1973) and in *Chara braunii, C. globularis* Thuill., *Nitella flagelliformis* A. Br. and *N. furcata* (Roxb. ex Bruz) Ag. by Sarma & Tripathi (1976a and b). Multiplication of chromosomes has also been recorded in *Chara braunii* when treated with acenaphthene by Sarma & Tripathi (1976b). Yoshida *et al.* (1972) report reduction in chromosome number in barley root-tip cells when subjected to chloramphenicol treatment, probably because of non-disjunction of chromosomes.

The significant cytological aberrations noted in the present study when treated with streptomycin include clumping of chromosomes (Fig. 4), micronuclei, degenerate and vacuolate nuclei (Fig. 2), anaphasic bridges (Fig. 3) and unequal distribution of chromosomes (Fig. 1). Srivastava & Sarma (1980), while studying the effect of streptomycin on the karyology of *Oedogonium gunnii*, have recorded chromosome breaks and fragments. Tanaka & Sato (1952) have found clotting, contraction and fragmentation of chromosomes along with the formation of micronuclei in *Tradescantia palludosa* after treating it with this antibiotic. Wilson (1950) records clumping of chromosomes in *Allium cepa* L. when treated with streptomycin. This drug has also caused fragmentation of chromosomes in the root-tip cells of *Vicia faba* L. as noted by Sharma & Bhattacharyya (1967).

It is interesting to record that cytological disruptions, encountered with streptomycin are almost identical to those induced by X-ray irradiations or by the action of mutagenic chemicals or by synthetic bioregulants like morphactin and chlorflurenol (Bhatnagar & Johri, 1987). The action of this antibiotic upon the mitotic cells appear thus to be mutagenic. It has potential to induce both minor

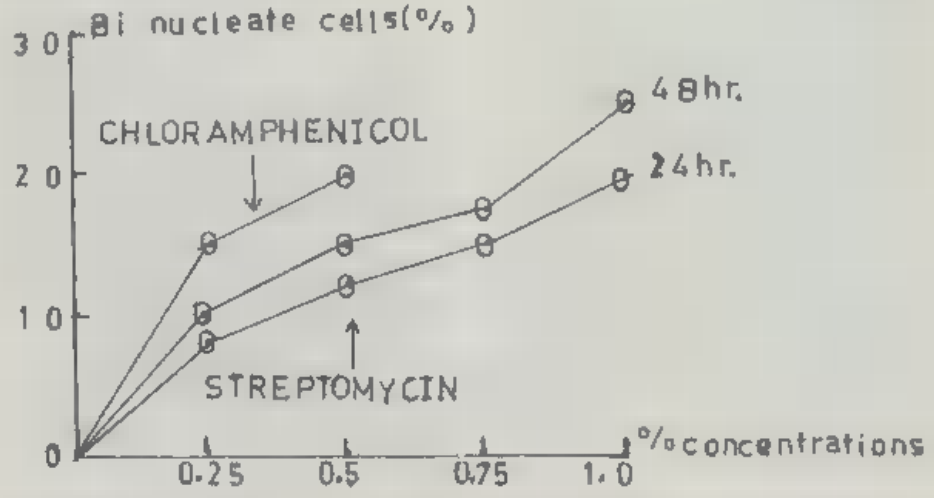


Figure 13: Effects of different concentrations of chloramphenicol and streptomycin on the percentage of bi-nucleate cells in *Chara braunii* Gm.

genetic recombinations as well as major genetic changes like translocation or polyploidy (cf. Tanaka & Sato, 1952; Wilson, 1950; Srivastava & Sarma, 1980).

Cytological abnormalities caused by chloramphenicol in the present study are degenerated nuclei, clumped metaphase plates (Fig. 8) and laggards (Fig. 11). Vedajanani & Sarma (1978) have reported frequent chromosome and chromatid breakages, anaphasic bridges, vacuolization and degeneration of nuclei, when *Spirogyra azygospora* is subjected to chloramphenicol treatments. Dot-type chromosomal fragments have been recorded by Yoshida *et al.* (1972) in barley roots while Shah (1975) opines that chloramphenicol inhibits protein synthesis and affects DNA synthesis in root-tip cells of *Vicia faba*. Parida & Manna (1967) mention its chromosome breaking activity on spermatocyte chromosomes of grasshoppers, probably because of its action on nucleoproteins of the chromosomes.

On the basis of the foregoing discussion, it is obvious that streptomycin and chloramphenicol are both mutagenic. Very low concentrations of chloramphenicol are able to induce major alterations like polyploidy. Minor aberrations like laggards, degenerated nuclei, clumping of chromosomes, etc. are caused by all the doses of the antibiotics employed in the present work. Chloramphenicol in lower doses is more effective than streptomycin.

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